

# Recent activation of the plaque immune response in coronary lesions underlying acute coronary syndromes

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## Abstract

**Objective**—To discriminate between chronic inflammation and acute activation of the plaque immune response in culprit lesions of patients with acute coronary syndromes.

**Design**—Retrospective study.

**Setting**—Tertiary referral centre.

**Subjects**—71 patients having coronary atherectomy were classified according to their ischaemic syndrome: stable angina (n = 23); stabilised unstable angina (n = 18); refractory unstable angina (n = 11); and acute myocardial infarction (n = 19).

**Main outcome measures**—Immunohistochemical measurement of interleukin 2 receptor (IL-2R) (CD25) positive cells expressed as a percentage of the total amount of (CD3 positive) T lymphocytes in frozen sections of atherectomy specimens.

**Results**—The number of lesions containing IL-2R (CD25) positive T cells increased with severity of the ischaemic coronary syndrome (stable angina, 52%; stabilised unstable angina, 77.8%; refractory unstable angina, 90.9%; acute myocardial infarction, 89.4%). The percentage of activated T cells (CD25/CD3 ratios  $\times 100$ ) increased in lesions associated with refractory unstable angina (7.8%) and acute myocardial infarction (18.5%), compared with those in lesions associated with either chronic stable angina (2.2%) or stabilised unstable angina (3.3%).

**Conclusions**—An increase in the percentage of IL-2R positive T lymphocytes in culprit lesions of patients with acute coronary syndromes indicates recent activation and amplification of the immune response within plaques. This may result in a burst of inflammatory products with tissue degrading and vasoactive properties and, hence, could initiate or accelerate the onset of an acute coronary event.

(Heart 1998;80:14-18)

**Keywords:** interleukins; T lymphocytes; acute coronary syndromes; atherosclerosis

Several insights into the pathobiology of atherosclerosis derive from the notion that the disease has many features of a chronic inflammatory response.<sup>1</sup> Pathological studies of human plaque tissue have shown that plaque inflammation is partly mediated by T cell immune responses.<sup>2</sup> Expression of activation markers on a subpopulation of T lymphocytes in plaque tissue strongly suggests antigenic stimulation during atherogenesis.<sup>2-5</sup> It is not known which antigens initiate the immune response, however, the capability of a subpopulation of plaque T cells to recognise antigenic determinants of oxidised lipids suggests a relation between lipids and plaque inflammation.<sup>6</sup>

Inflammation is associated with plaque rupture or erosion that precedes the onset of acute coronary syndromes. Postmortem examination has shown that thrombosed coronary plaques in infarct related arteries contain an inflammatory infiltrate at the underlying site of rupture or erosion, with cellular characteristics already described.<sup>7,8</sup> Moreover, coronary atherectomy specimens from culprit lesions of patients with symptomatic coronary artery disease have increased numbers of macrophages and T lymphocytes with severity of the ischaemic syndrome.<sup>9,10</sup> A steady and progressive increase in the number of inflammatory cells may gradually induce a retarded destabilising effect in the plaque, which may eventually lead to plaque erosion or rupture. Alternatively, recent (immune) activation of the inflammatory response, with secretion of cytokines, vasoactive molecules, and proteolytic enzymes, may give rise to rapidly progressive plaque destabilisation. The potential relation between acute coronary syndromes and recent activation of the plaque inflammatory response has not been investigated. Interleukin 2 receptors (IL-2R) on T lymphocytes are expressed shortly after stimu-

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Accepted for publication  
30 October 1997

Table 1 Histopathological analysis of 71 atherectomy specimens

Group	Number	Total (SEM) area (mm <sup>2</sup> )	Fibrous tissue (% of lesions)	Thrombus (% of lesions)	Atheroma (% of lesions)
1 Chronic stable angina	23	9.0 (1.0)	100	26	52
2 Unstable angina (Braunwald class I, II)	18	9.29 (1.02)	100	61	78
3 Unstable angina (Braunwald class III)	11	8.56 (1.19)	100	82	82
4 Acute myocardial infarction	19	10.82 (0.93)	100	100	94.7

lation and persist for only a few days. The presence of IL-2R may, therefore, serve as a marker for recent T cell activation in a tissue immune response.<sup>11</sup> This investigation aimed to assess the number of T cells and the percentage of IL-2R positive T cells in coronary atherectomy specimens from patients with various ischaemic coronary syndromes and to correlate the results with severity of the disease.

## Methods

### ATHERECTOMY SPECIMENS

Coronary atherectomy specimens were obtained from 71 patients who underwent directional coronary atherectomy at the Academic Medical Center, Amsterdam, for a single primary lesion (restenosis specimens, vein graft specimens, and specimens from patients who required multiple interventions at the same time were not included).

Patients were selected for directional coronary atherectomy on a strictly defined angiographic criterion: a proximally located eccentric culprit lesion in a non-tortuous coronary artery of more than 3 mm in diameter. Patients were prospectively classified into four groups: chronic stable angina (duration of more than two months, classified according to the Canadian Cardiovascular Society, classes I to III:  $n = 23$ )<sup>12</sup>; "stabilised" unstable angina (new onset or accelerated angina or angina at rest but not within the pre-

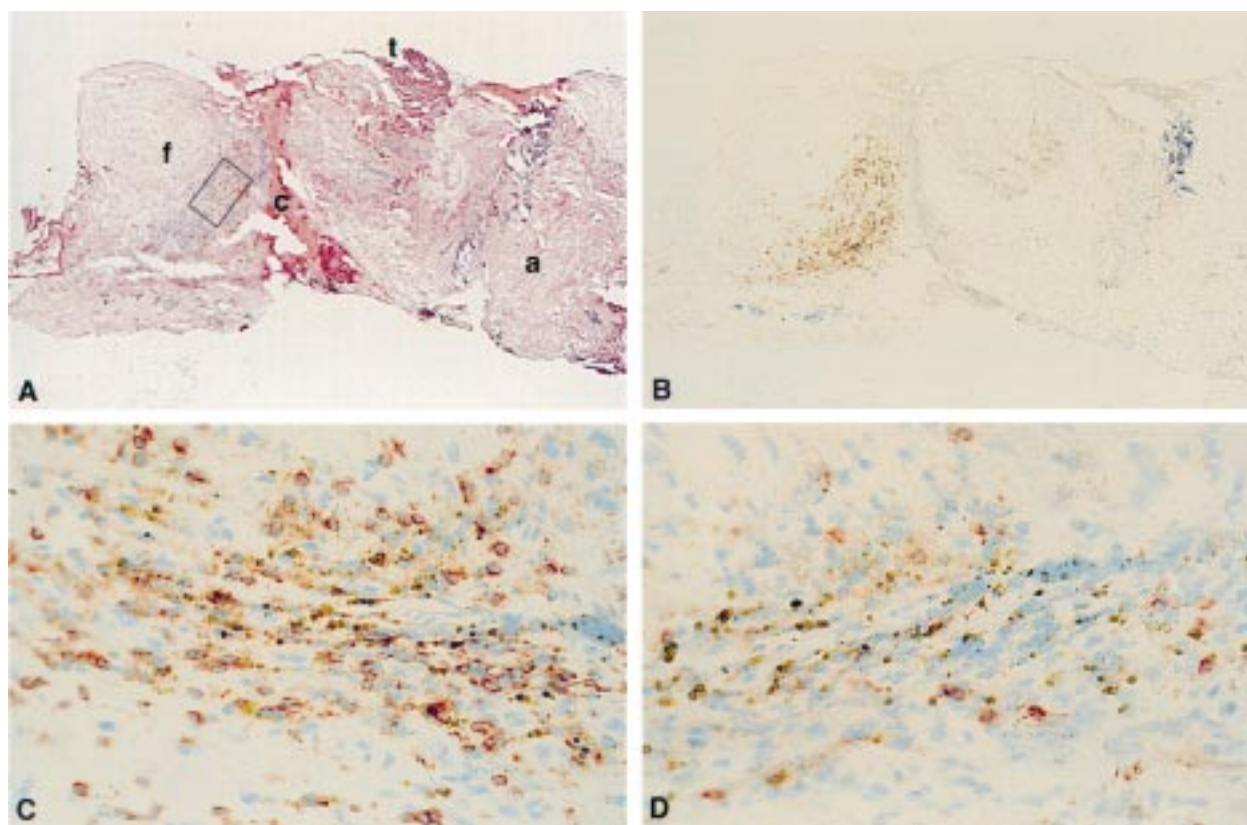
ceding 48 hours, Braunwald class I, II:  $n = 18$ )<sup>13</sup>; "refractory" unstable angina (angina at rest, within 48 hours, Braunwald class III:  $n = 11$ )<sup>13</sup>; and acute myocardial infarction (angina at rest, persisting for more than 30 minutes, accompanied by electrocardiographic signs of transmural ischaemia and presentation within four hours after the onset of symptoms:  $n = 19$ ).

### LIGHT MICROSCOPY

Immediately after atherectomy the tissue specimens were carefully oriented along their longest axis and frozen in liquid nitrogen. Frozen serial sections (5  $\mu$ m) were cut and two sections were stained with haematoxylin and eosin and elastic van Gieson for histomorphological analysis. Adjacent sections were mounted for immunohistochemical examination.

### IMMUNOHISTOCHEMISTRY

A three step indirect peroxidase technique on acetone fixed frozen sections was used as previously described.<sup>14</sup> The primary antibodies were anti-CD3 (immunoglobulin G1 isotype, dilution 1/20; Becton Dickinson, San Jose, California, USA), which was used to visualise the entire T cell population, and anti-CD25 (Dako IL-2R, code M731, dilution 1/100, immunoglobulin G1 isotype; Dako, Glostrup, Denmark), which was used to detect IL-2R on activated lymphocytes. Sections were counterstained faintly with haematoxylin. Lymphoid



**Figure 1** (A) Haematoxylin and eosin stained section of a specimen from a patient with Braunwald type III unstable angina showing fibrocellular tissue (f), thrombus (t), atheroma (a), and a fresh clot related to atherectomy (c). (B) Anti-CD3 stained section adjacent to panel A shows a cluster of T cells and scattered immunopositive cells throughout the fibrocellular tissue. (C) Higher power magnification of the boxed area of (A) stained with anti-CD3, showing the ring shaped staining pattern of all T cells (CD3 positive). (D) Higher power magnification of the boxed area of (A) showing a subpopulation of IL-2R positive cells stained with anti-CD25. The yellow granules are ceroid pigment, an end product of lipid oxidation.

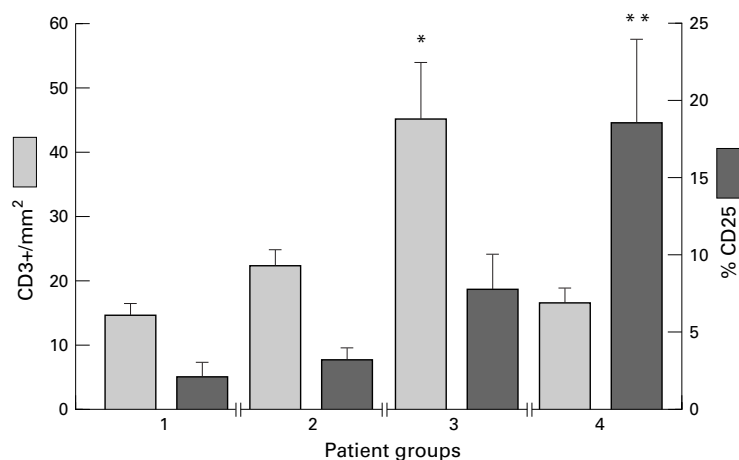


Figure 2 Mean (SEM) numbers of T cells (CD3 positive) per mm<sup>2</sup> atherectomy tissue and percentages of activated (CD25 positive) T cells (CD25/CD3 × 100) for each patient group. Group 1, stable angina; group 2, stabilised unstable angina; group 3, refractory unstable angina; group 4, acute myocardial infarction. \* $p_{1-3}$ ,  $p_{2-3}$ , and  $p_{3-4} < 0.05$ . \*\* $p_{1-4}$  and  $p_{2-4} < 0.05$  (post hoc test).

tissue (human palatinal tonsil) served as a positive control for both stains. Negative controls consisted of sections incubated according to the same technique, but the primary antibody was substituted with an irrelevant monoclonal reagent of similar isotype.

#### MORPHOMETRY

Total tissue areas of immunostained sections were measured (mm<sup>2</sup>) by computer aided planimetry. Numbers of CD25 positive and CD3 positive cells were counted in the entire tissue sections and expressed as cells per mm<sup>2</sup> tissue. These data were used to calculate the percentage of activated T cells (CD25/CD3 × 100) for each section. Pathological analysis (histopathology and morphometry) was performed unaware of the patient's clinical diagnosis.

#### STATISTICAL ANALYSIS

Data are expressed as means (SEM). Analysis of variance and a post hoc Scheffé test were used for multiple comparisons of parametric data (morphometric results in four patient groups).  $p < 0.05$  was considered significant.

#### Results

Histopathological analysis showed that all specimens contained fibrous tissue, either fibrocellular or fibrosclerotic, or both. The number of lesions containing thrombus and atheroma was highest in patients with refractory unstable angina or acute myocardial infarction. There were no differences in total tissue areas of sections among the four patient groups (table 1).

Immunohistochemical examination showed that all lesions contained CD3 positive T cells, which were frequently seen in clusters in fibrocellular tissue and the border zones of atheromas (fig 1). Variable numbers of scattered T cells were also present throughout the tissues. Thrombus and extracellular lipid fragments (atheroma) contained few, if any, lymphocytes. Medial fragments were devoid of T cells. The mean (SEM) numbers of CD3 positive T cells

per mm<sup>2</sup> increased from patient group 1 (stable angina: 14.7 (1.8)) to groups 2 (Braunwald class I, II: 23.1 (2.6)) and 3 (Braunwald III: 45.1 (8.8)). In patient group 4 (acute myocardial infarction), however, the mean (SEM) number of CD3 positive cells was lower (16.5 (2.3)) ( $p_{1-3}$ ,  $p_{2-3}$ , and  $p_{3-4} < 0.05$ ).

CD25 positive T cells were also found in clusters, although in much fewer numbers. Moreover, they were present in only a few lesions. Fifty two per cent of the total number of lesions of patients with chronic stable angina contained CD25 positive T cells. Patients with unstable angina (group 2: 77.8% of lesions and group 3: 90.9%) and myocardial infarction (89.4%) had a much higher incidence of lesions with CD25 positive T cells. The relative number of activated T lymphocytes (CD25/CD3 ratios calculated for each specimen) increased from patient groups 1 (0.022 (0.009)) and 2 (0.033 (0.007)) to groups 3 (0.078 (0.003)) and 4 (0.185 (0.054)) ( $p_{1-4}$  and  $p_{2-4} < 0.05$ ) (fig 2).

#### Discussion

Increased numbers of inflammatory cells, including macrophages, T lymphocytes, and mast cells, in atherosclerotic plaques are associated with severity of the acute coronary syndrome.<sup>9-10</sup> The present study also shows an increase in recent activation of the T cell response. Other studies have reported the presence of several activation markers on T cells in human atherosclerotic plaques. A large proportion of T cells within randomly sampled plaques present the very late activation antigen VLA-1 and HLA-DR molecules, whereas relatively low numbers express IL-2R.<sup>5</sup> This suggests long term low level activation in atherosclerosis.<sup>15</sup> These studies, however, were carried out on randomly selected, mostly uncomplicated plaques, and none relates these findings to clinical status.<sup>3-5</sup> In this study the percentage of IL-2R positive (CD25 positive) T cells increased in atherectomy specimens obtained from patients with refractory unstable angina (7.8% of the total number of (CD3 positive) T cells) or acute myocardial infarction (18.5%) compared with the relatively low percentages in specimens from patients with stable angina (2.2%) or stabilised unstable angina (3.3%).

Expression of IL-2R is an important marker for T cell activation, as it indicates recent stimulation of these cells in a cell mediated immune response. IL-2R appears on the surface of T cells shortly (within 2–24 hours) after cytokine stimulation by macrophages and persists for only a few days after diminution of the stimulating agents.<sup>16</sup> During this period a variety of inflammatory mediators are produced, particularly by cytokine activated macrophages and T cells themselves. A causal role for the local burst of inflammatory activity and the onset of acute syndromes seems likely, as inflammatory products secreted during amplification of the immune response may have detrimental effects on stability of the fibrous skeleton and integrity of the plaque endothelium and are also capable of increasing local

vasomotor activity of the vessel wall.<sup>17, 18</sup> It seems therefore that T cell activation is another feature of the complex of biological processes associated with plaque destabilisation.

Raised levels of systemic markers for inflammation, such as C reactive protein,<sup>19, 20</sup> and activated circulating monocytes<sup>21</sup> have been reported in patients with severe unstable angina. Recently, Sernerer and colleagues<sup>22</sup> found increased numbers of HLA-DR positive T cells and increased levels of soluble IL-2R (S IL-2R) in the serum of patients with unstable angina. These variables gradually decreased over a period of eight weeks after the coronary event, indicating that acute T cell activation can be detected in serum around the time of onset of coronary syndromes. Systemic markers for lymphocyte activation, however, may not necessarily relate to the situation in the atherosclerotic plaque, and may arise from another inflammatory (infectious) disease.<sup>23</sup>

The relatively low number of total (CD3 positive) T cells in specimens from patients with acute myocardial infarction was surprising. This finding probably relates to the substantially larger amounts of thrombus, practically devoid of T cells, that were found. The ratio of activated T cells versus the total number of T cells, the prime interest of this study, however, is not affected by thrombus.

Immunocytochemical detection of IL-2R on T lymphocytes is considered to be a strong indication that recent antigenic stimulation has occurred. Indeed, the microenvironment of atherosclerotic plaques contains additional components necessary for antigenic stimulation—for example, HLA-DR positive macrophages, which potentially may serve as antigen presenting cells,<sup>2</sup> and sets of costimulatory molecules and their ligands on macrophages and T cells.<sup>24</sup> Moreover, in situ studies of human plaque tissue have shown that cytokines produced by inflammatory cells include  $\gamma$  interferon and interleukin 12, but virtually no interleukin 4.<sup>25, 26</sup> These findings indicate a preference for a cell mediated immune response in atherosclerotic plaques.

Recognition of increased recent activation of the immune response in plaques underlying acute coronary events emphasises further the need to identify those antigens responsible for triggering the process. Of several potential antigens that have been suggested, including heat shock proteins,<sup>27, 28</sup> neoantigens on cytomegalovirus infected cells,<sup>29</sup> chlamydial antigens,<sup>30</sup> and lipid derivatives,<sup>6, 31, 32</sup> antigenic determinants of oxidised low density lipoproteins (oxLDL) are of particular interest. So far, oxLDL is the only antigenic structure capable of stimulating human plaque derived T lymphocytes in vitro.<sup>6</sup> Moreover, cellular analysis of atherosclerotic lesions in apolipoprotein E knockout mice, performed by the same researchers, has shown participation of CD25 positive, CD4 positive cells in lesion formation in these genetically hypercholesterolaemic animals. The authors speculated that there might be a direct link between accumulation of cholesterol in the vessel wall and activation of T cells, possibly by autoimmune responses to

modified lipoproteins.<sup>32</sup> Interestingly, we regularly observed clusters of lymphocytes in close proximity to ceroid pigments, which are considered to be end products of lipid oxidation in plaque tissue (fig 1). If derivatives of oxLDL are responsible for activation of recent immune responses in plaques, either solely or in combination with other antigens, then patients with symptomatic coronary artery disease should be managed from a different perspective. Lipid lowering strategies may be beneficial not only because they can decrease the lipid mass of plaques as a long term effect, but also because they can reduce critical levels to induce a “burst” of recent inflammation and, hence, the incidence of acute coronary events.<sup>19, 20</sup>

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